

Using Phage as A Highly Specific Antibiotic Alternative Against *Methicillin Resistance Staphylococcus aureus* (MRSA)

Fatemeh Rezaei¹, Ahmad Nasser^{2*}, Farid Azizi Jalilian⁵,
Zack Hobbs³ and Reza Azizian^{2,4}

¹Department of Virology, TarbiatModares University, Tehran, Iran.

²Student Research Committee, Ilam University of Medical Sciences, Ilam, Iran.

³The Evergreen State College, Olympia, Washington, USA.

⁴Research & Developing Center, Mahan Gene Pajoh (Poyesh), Tehran, Iran.

⁵Department of Microbiology, Ilam University of Medical Science, Ilam, Iran.

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Misuse of antibiotics in humans and animals often leads to the development of multidrug resistance (MDR) bacteria. Resistance can occur within a few years of novel antibiotics being introduced. Lytic bacteriophage (phage) are a kind of virus that undergo a cyclical lifestyle wherein they infect and replicate through the use of a bacterial host cell and cause cell lysis. Phage recognizes specific receptors on their host cell to attach to, insert their DNA and take over their host's molecular machinery. These receptors only exist on the surface of specific bacterial host cells and are often not present on other non-specific bacteria and not especially on the surfaces of eukaryotic cells. The mechanisms by which phage can destroy bacteria are different from antibiotics; phage can lyse MDR resistant bacteria without being affected by hydrolytic enzymes or ribosomal variations that's mean unlike drug resistance mechanism which bacteria can destroy the drug before can enter the bacteria cell, the phage not effected with such enzyme.

Key words: Antibiotics, MRSA, Human and Animals.

Bacteriophages (phages) are viruses that insert their genomes into bacterial cells, rapidly taking control of the host's molecular machinery and produce progeny that are released by lysis of the bacterium. Phage therapy involves using phages as a treatment or prophylaxis application against infectious diseases caused by bacteria. The worldwide prevalence, persistence and incurability of pathogenic multidrug resistant bacteria is a serious public health concern that will bring about the post-antibiotic era. This result is primarily from the extensive use of antibiotics that puts selective pressure on bacteria to develop mechanisms of resistance¹. Phage were first discovered separately by Fredrick Twort and Félix

d'Herelle in the early 20th century and later used heavily among Eastern Europe countries and the former Soviet Union for treating and preventing bacterial infectious diseases[2-4]. In more recent years an interest in phage therapy has been resurrected as a potential option to deal with MDR bacteria infections.

MRSA

Staphylococci are gram-positive bacteria that cause various infectious diseases in humans and animals. *Staphylococci* are classified using different methods, one of the main categories on the basis of coagulase. Coagulase is a major factor that causes blood clotting in the proximity of the bacteria and bacteria lacking in these factors often make poor pathogens. Accordingly, the only species of Coagulase-positive *Staphylococci* in this category is *S. aureus*. *S. aureus* initially was susceptible to *Penicillin*, but with time and indiscriminate use of antibiotics, resistant strains

* To whom all correspondence should be addressed.
Tel.: +989166438900;
E-mail: a.nasseri27@gmail.com

emerged along with ones that became resistant to more recent generations of β -lactam antibiotics. *Methicillin* was among the later generation drugs and became commonly used after the emergence of *Penicillin*-resistant *S. aureus*^{5,6}. Two subsets of *S. aureus* strains are further distinguished based on resistance and susceptibility to *Methicillin*; MRSA (*Methicillin Resistance Staphylococcus aureus*), and MSSA (*Methicillin Sensitive Staphylococcus aureus*) (Fig. 1).

Diseases caused by *S. aureus* include endocarditis, wound infection, carbuncle, toxic shock syndrome, osteomyelitis, etc. These bacteria produce a toxin that damage cells as well as being able to directly attack the cell to cause apoptosis using a *Fibronectin Binding Protein*⁷. Drug resistance in MRSA is the result of a *mecA* gene that produces *Penicillin-Binding Protein* (PBP) with a lower affinity⁸. The PBP is a peptidoglycan synthesis protein and involved in the synthesis of peptidoglycan, which is the major component of bacterial cell walls.

Phage

Nearly 10^8 phage particles per ml are present in the oceans and an estimated 10^{30} phage particles exist in the world. Phages can be divided based on features such as host range, absence or presence of an lipid envelope, physical characteristics (size and morphology of capsid and tails, if present), resistance to organic solvents, genome composition (single or double-stranded DNA or RNA) and if they have antigenic properties^{9,10}. Based on classification by the International Committee of Taxonomy of Viruses (ICTV) a vast majority of phages are in a one category called *Caudovirales*⁹, have one of three tail morphologies and ads DNA genome packaged a head-full into their capsid¹¹. Phages are further divided into three types based on their life cycle: lysogenic, lytic and chronic¹². The genome of a temperate phage, once inserted into the bacterial cytoplasm, integrates into the host chromosome and can undergo a dormant state, even be replicated along with the host chromosome during binary fission and at a later time excise from the chromosome to form phage particles and utilize the lytic cycle¹³. Obligatorily lytic phages however inject their genome into the cytoplasm of the bacteria, hijack the cell and immediately convert it into producing progeny and shortly (or eventually

a few hours later which is the case with T4-like phages due to lysis inhibition) releases them by destroying the bacteria through lysis. Chronic phage can replicate and release their progeny from the host without compromising the viability of their host. Phages can survive outside the host cell but utilization of the host is required for replication¹⁴. Phage specificity can be used to further characterize bacterial strains through a method called phage typing^{15, 16}.

There are several problems to identifying phages one of which is that phages cannot be cultured outside of a host¹⁷. Phage particles will be comprised of a tail, a filamentous tube or polymorphous structure¹¹. The composition of a *Caudoviridae* includes a capsid, disk plates that make a tail sheath, a base plate and a spike for *Siphoviridae* or multiple tail fibers for *Podo-* and *Myoviridae*. In addition to phage therapy, phage can be used for industrial purposes, including phage display, detection of pathogenic bacteria via luminescence¹⁸, provide bactericidal endolysin proteins that clear if not mitigate the prevalence of pathogens from food products²⁰, enhanced food safety applications in agricultural and aquaculture settings, as a prophylactic and in hospitals to control biofilm-forming pathogens and common bacterial infections associated with hospital visits¹⁹.

Advantages of phage therapy

(A) Effective against MDR bacteria because they use significantly different mechanisms in comparison to antibiotics; (B) Phages are capable of evolving/mutating therefore, they can respond to phage-resistant bacteria; (C) Shifting of target organisms does not occur because it has high specificity and secondary infections are avoided²¹; (D) Phages or their protein products, such as holin and lysozyme, do not negatively affect eukaryotic cells^{22, 23}; (E) Phages can replicate at the site of a bacterial infection; (F) Phage-resistant bacteria are likely susceptible to other phages²⁴.

Classification of phage and infection mechanism:

Phages are classified into 13 families according to nucleic acid type, morphology and the presence or absence of an envelope or lipid layer²². Most phages have a tail and are further classified into three families according to the morphological features of a tail: *Myoviridae* (a contractile tail most commonly found in T-even phages), *Siphoviridae* (long, flexible, non-contractile tail seen in T5) and

Podoviridae (extremely short tail; e.g., T7)^{25,26}. Phage infections function much like other viruses wherein the first step is to make contact with a receptor, usually a protein or sugar component on the bacterial surface. Phages are able to absorb to specific bacterial species and even to specific strains within a species; or genera within the same gram type which are called polyvalent phages²⁷. The second step (for lytic phages specifically) is the injection of DNA into the bacterial cytoplasm,

use the host RNA polymerase to express early mode genes from the phage genome, take over of the host by either degrading the host genome or ejecting it from the cell, replicate the phage genome, and synthesized multiple middle and late genes that encode proteins such as the capsid and tail, followed by the genomic DNA being packaged into the capsid, resulting in completed phage. In the last step, phages produce the endolysin that destroy the peptidoglycan of the cell wall allowing phage progeny to be released²⁸.

Table 1. Phage therapy: Pros and Cons

Researcher(s)	Title	Year	Result
Bruynoghe R. and Maisin J.	Essai de thérapeutique aumoyen du bacteriophage	1921	Phage treated <i>Staphylococcal</i> skin disease by injecting the phage near the site of the infection.
Smith, H. W., and Huggins M. B.	Successful treatment of experimental <i>Escherichia coli</i> infections in mice using phages: its general superiority over antibiotics	1982	Successful treatment of <i>E. coli</i> infections in mice without collateral damage.
Smith, H. W., and M.B. Huggins.	Effectiveness of phages in treating experimental <i>E. coli</i> diarrhea in calves, piglets and lambs	1983	Used single doses of specific phage to treat diarrheal <i>E. coli</i> strains, reduced and/or stopped the diarrhea.
S 'Lopek S. et al	Results of bacteriophage treatment of suppurative bacterial infections in the years	1981–1986	Phage were used to treat 518 patients with antibiotic resistant bacterial infections; the success rate of curing patients was between 75–100%.
Bogovazova, G. G., et al.	Immunobiological properties and therapeutic effectiveness of preparations from <i>Klebsiella</i> bacteriophages	1992	Demonstrated their phage to be safe and effective against treating <i>in vivo</i> <i>Klebsiella</i> infections.
Soothill, J. S. et al.	Bacteriophage prevents destruction of skin grafts by <i>Pseudomonas aeruginosa</i>	1994	Phage used as a bio-control against <i>P. aeruginosa</i> infections.
Thiel K.	Old dogma, new tricks 21st Century phage therapy	2004	Demonstrated how therapeutic phages can be produced inexpensively
Gill J. J. et al.	Efficacy and Pharmacokinetics of Bacteriophage Therapy in Treatment of Subclinical <i>Staphylococcus aureus</i> Mastitis in Lactating Dairy Cattle	2006	This study showed the ability of daily dose of lytic staph phage K to eliminate bovine <i>S. aureus</i> intra-mammary infections during lactation in 24 cows.
Trigo G. et al.	Phage Therapy is Effective against Infection by <i>Mycobacterium ulcerans</i> in a Murine Footpad Model	2013	<i>Mycobacteriophage</i> D29 was evaluated for therapeutic efficacy in footpad mice

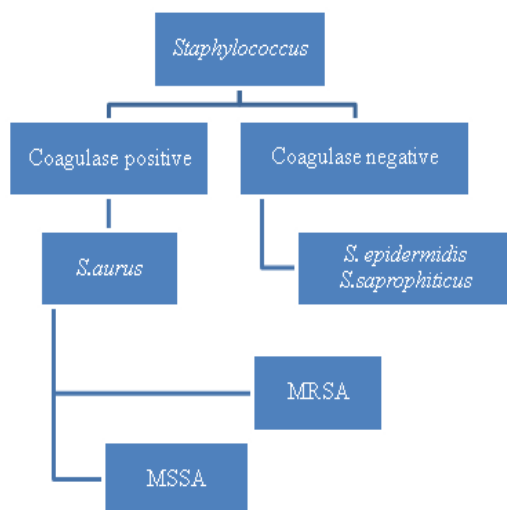


Fig. 1. Differentiation of *S. aureus* based on Coagulase

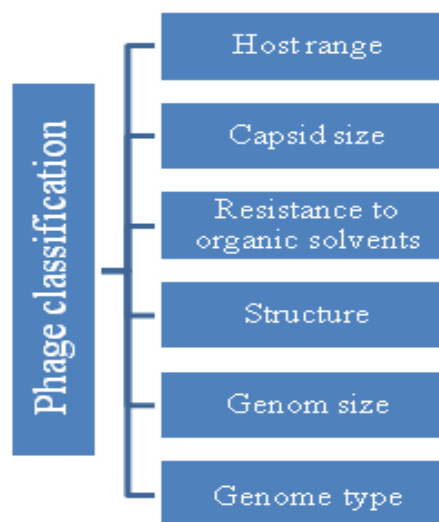


Fig. 2.

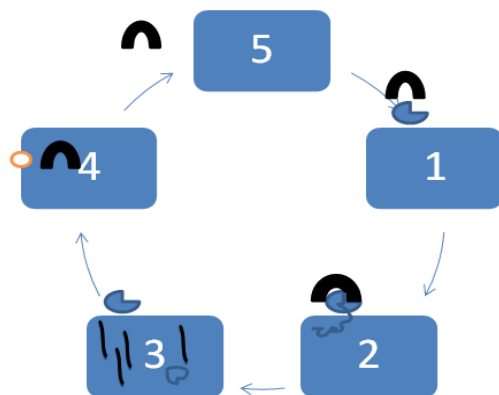


Fig. 3. Mechanism of phage-induced lysis of the bacteria: 1) Phage detects specific receptors and adsorbs to them. 2) In the second step, the adsorbed phage injects its genome into the bacterial cytoplasm. 3) Host molecular machinery recognizes promoters on phage genome causing transcription and translation of protein products such as capsid and enzymes meanwhile the phage genome is replicated. 4) Assembly of completed phage particles, lysis of bacteria with holin and exit out of the cell by diffusion. 5) Attach to new host and repeat cycle

Phage and genetic engineering

Most gram-negative bacteria have extracellular lipopolysaccharide components that are often shared amongst different strains. Through the use of genetic engineering, we could modify the properties of a phage's tail fibers that could allow it to bind to one or more of the shared components

found in most lipopolysaccharide of gram-negative bacteria of interest resulting in a wider host range. In a study conducted by Lorena Rodríguez-Rubio three different proteins used between lysostaphin and HydH5 resulted in increased performances of the 2 proteins compared to the wild type and caused lysis of the bacteria in plate and reduce the opaqueness of bacteria in liquid media²⁹.

Phage therapy vs. Antibiotic therapy against MRSA

The use of phage as an antimicrobial agent with similar efficacy to an antibiotic is one of the most exciting aspects in treating infections. A number of studies have shown the use of phage has no direct, negative effects on eukaryotic cells and the efficiency of phage can in some cases be greater than antibiotics. In addition, the problematic, disease-causing bacteria can be destroyed with no or minimal effect on neighboring and essential microbiota. Smith et al. showed that a single intramuscular dose of one anti-K1 coliphage was more effective for treating mice challenged with *E. coli* intramuscularly than multiple intramuscular doses of Tetracycline, Chloramphenicol or Trimethoprim plus Sulfafurazole³⁰. Chibani-Chennouf et al. showed that all of the administered phage passed through the gastro in testinal tract in adult mice and destroyed a diarrheal *E. coli* strain³¹. Matsuzaki et al. used four types of phage to treat 72 strains of MRSA (Methicillin Resistance

Staphylococcus aureus) and showed that injections of 8×10^8 bacteria intra-peritoneal, caused bacteremia and eventual death in mice but when administration simultaneously with purified phage ØMR11 (MOI ≥ 0.1) they suppressed *S. aureus*-induced lethality. High-doses of phage ØMR11 used on uninfected mice showed no adverse effects³². Międzybrodzki *et al* did a comparison between the cost of a phage therapy and antibiotic therapy and showed that the use of phage therapy in MRSA infections was less expensive to use than antibiotics such as *Vancomycin*, *Linezolid*, *Teicoplanin* and *Chinupristin + Dalfopristin*³³. Clem attempted to use Staphphages to eliminate numerous MRSA strains and showed that many of their phages could not infect some of the strains and suggested a plausible phenomenon relating to the surface antigen properties such as clumping factor A, clumping factor B, *Fibronectin Binding Protein A*, *Fibronectin Binding Protein B*, *collagen adhesion*, *SdrC*, *SdrD*, *SdrE*, Protein A and *Methicillin* resistance surface proteins³⁴. Gu *et al* used the phage endolysine (LysGH15) as a prophylactic to protect mice against the MRSA infections. Their results demonstrated that 50 µg of LysGH15 was sufficient to protect mice against injections at double the minimum lethal dose of MRSA when administered 1 hour prior to the bacterial challenge³⁵. S. O'Flaherty *et al* discovered and tested two novel lytic phages (DW2 and CS1) against their entire personal collection of *Staphylococci* species that cause mastitis-associated infections and demonstrated their phages capabilities of being used as a prophylactic³⁶. According to the above studies we concluded that the use the phage is often cheaper and promising for the treatment of MRSA infections. Data shown in Table-1.

Problems with Phage therapy and possible solutions

- A) Most phage have limit detection of the host, so that a narrow range of hosts ability to identify with phage. A possible solution could be to use polyvalent phages that can identify a wide range of bacteria³⁷.
- B) The presence of cellular debris and other by products from the host bacteria in phage preparations can cause negative immune responses. Using modern technology such as density gradient centrifugation can eliminate or greatly mitigate a patient's

exposure to these elements³⁸.

- C) Phage clear from the body rather fast if their host is not available, for more persistence can be used strains that delay detected and cleared by the immune system³⁹.

CONCLUSION

Numerous studies have used phage as an antimicrobial agent and have shown them to be effective at destroying target bacteria^{33, 40-43}. Phage are highly specific and are only capable of lysing bacteria and have not been demonstrated to cause lysis or have direct negative immunological effects to eukaryotic cells⁴⁴. Phages specificity is due to their tail fibers' ability to bind to specific antigens on their host that aren't present on other non-target bacteria. In other words the use of phage to attack problematic bacteria is beneficial and not likely to have any negative effects on normal microbiota; an effect often not seen in conventional broad-spectrum antibiotics. Another advantage of phage is that they can replicate at the site of infection and easily overcome and out number the bacteria after a number of infection cycles. The likelihood of a naturally occurring phage being able to infect a eukaryotic cell is highly unlikely and probably even impossible without the extensive use of genetic engineering in a lab setting. The receptors on the outer membrane of eukaryotes are significantly foreign in comparison to those of bacteria, the phage wouldn't be able to penetrate the significantly thicker cell membrane, the inserted genome likely wouldn't survive within the cytoplasm as well take over the molecular machinery of the host within the nuclei⁴⁵.

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